QUANTITATIVE EFFECTS OF VITAMIN A DEFICIENCY ON THE SPINAL AND SYMPATHETIC GANGLIA

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Since lesions of the nervous system due to avitaminosis were first reported by Mellanby ('26) there have been numerous papers dealing with the degenerative changes following experimental deprivation of the various vitamins in animals. These papers have reported several different types of effects, varying from moderate swelling of nerve fibers to definite degeneration, haemorrhage into various parts of the nervous system, and irritative or inflammatory changes not definitely degenerative in character. Many of the reports are contradictory. Most of the studies have been made with the usual neurological stains, such as Marchi, Speilmeyer's, and Sudan III. Thus Grinker and Kandel ('33) studied nerves from vitamin A deficient rats but found no changes in the nerve fibers. Hughes, Aubel and Lienhardt ('28) reported finding degeneration in cows, chickens and pigs. Aberle ('34) described extensive degeneration in brachial and sciatic nerves in rats on vitamin A deficient diets. Mellanby ('34) reported degeneration in dogs in vitamin A deficiency. His observation of degeneration in the posterior roots of spinal nerves and its absence in anterior roots led him to the hypothesis that the xerophthalmia which occurs in this disease is due to loss of sensory nerve supply. He also assumed a special selective action in the case of afferent fibers while efferent fibers were not affected. This does not accord with the observed symptoms of deficiency. These have been described by several investigators, including ourselves (Sutton, Setterfield and Krauss, ('34), Setterfield and Sutton, ('35), using the polarized light technique. This has been corroborated by Lee and Sure ('37). The flaccid paralysis and muscle atrophy accompanying this avitaminosis indicates destruction of efferent nerve fibers to the muscles affected. The loss of sense of pain and touch which occurs at the same time is explained by the observations on nerve fibers already published by us and by the results of the present study.

In this paper we wish to present further evidence that vitamin A deficiency causes definite and measurable destruction of peripheral nervous tissue sufficient to explain the loss of function already noted. Reference should be made to our previous publications for the roughly quantitative observations of degeneration in nerve fibers.

METHODS AND MATERIALS

In the present study we have made total cell counts of certain of the spinal ganglia of normal control and vitamin A deficient white rats[1]. The serial sections of the ganglia were stained with haemotoxylin and eosin and counted at a magnification of 900 diameters. A cross-hatched disc in the ocular made possible accurate counting of the cells. The nucleoli only in each section were counted since the cells are so large that they appear in several successive sections.

For this study forty-three ganglia were used, these being the second, third and fourth lumbar ganglia in all cases. They were distributed as follows: three normal second lumbars; three normal third lumbars; five normal fourth lumbars; eleven deficient second lumbars; nine deficient third lumbars and twelve deficient fourth lumbars. Control and experimental animals were litter mates and all the experimentals had been on the deficient diets from 55 to 64 days. All showed symptoms of paralysis (flaccid) and loss of sensation of pain and touch though in varying degrees. All were near death at the time of cancellation.

RESULTS AND COMMENT

Little is known as to the usual or normal number of cells in any spinal ganglion of any animal. A few studies have been made, notably that of Hatai ('02) on the white rat. In trying to determine the normal number of cells in the spinal ganglia at different ages he counted the cells of the sixth cervical, fourth thoracic, and second lumbar at four different ages. The rats used ranged from 10.3 grams weight to 167 grams. His figures are averages of each ganglion at the four ages used. It is of interest at this point that his average of the counts of the second lumbars differs by only 130 cells from our average given below. This close correspondence gives greater confidence that our counts are substantially correct.

Nerve cells in vitamin A deficiency show characteristic degenerative changes preceding their actual death and disappearance. In about the order of their occurrence these include gradual loss of staining qualities, shrinkage, pyknotic nuclei, eccentric nuclei, loss of the typical concentric arrangement of the cytoplasm, indistinct cell outlines, fading and disappearance of the nuclear membrane, and finally loss of the nucleolus. At about this time the cell is damaged beyond recovery.

[1]The diet used in this experiment was the standard U.S.P. XI, except that the casein was not extracted but contained a minute amount of vitamin A. Control animals received an adequate supplement amount of cod liver oil.

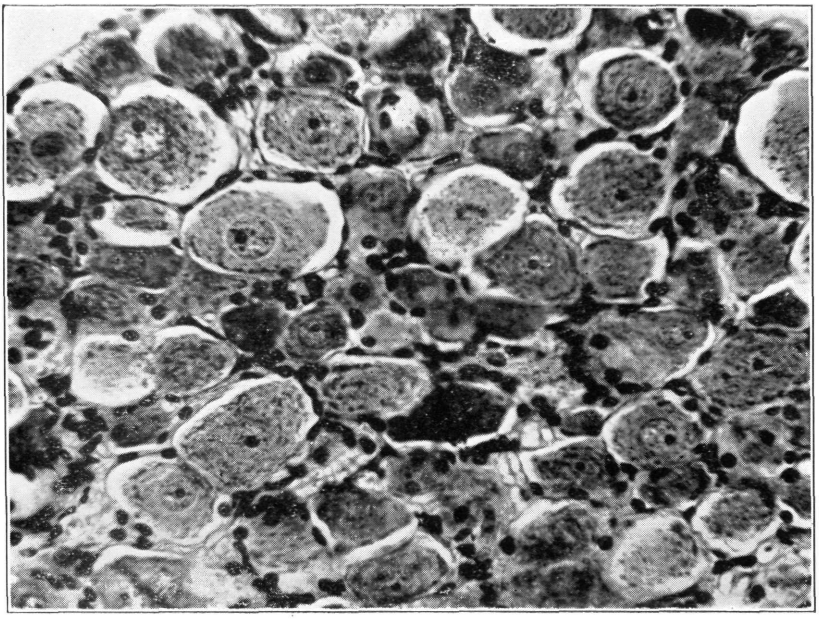

FIG. 1. Section of normal spinal ganglion of white rat. × 900.

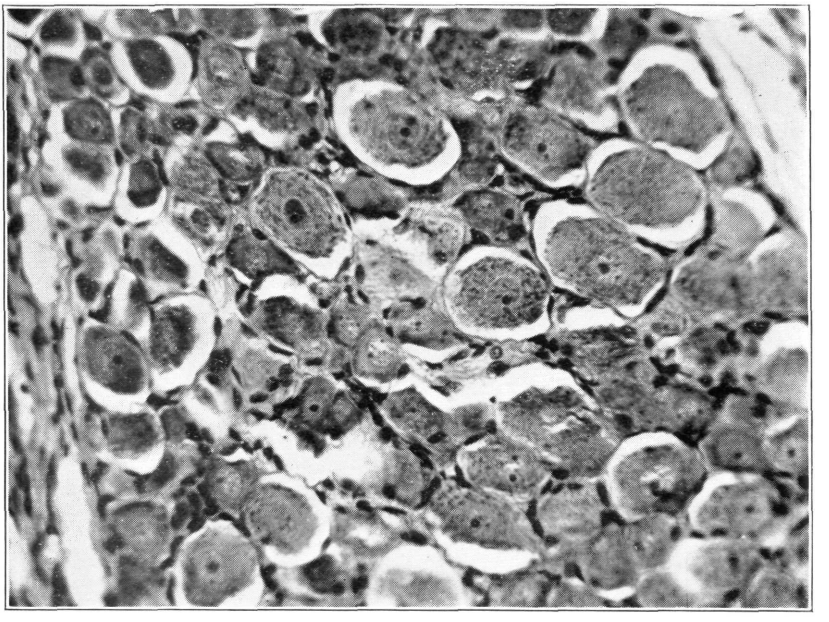

FIG. 2. Section of spinal ganglion of a vitamin A deficient white rat. Note extensive shrinkage and indistinct cell and nuclear outlines. × 900.

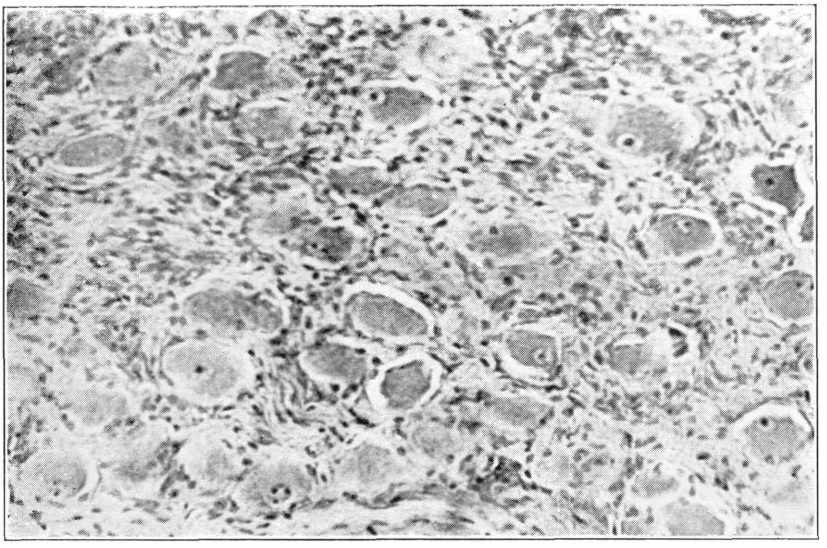

FIG. 3. Section of normal inferior cervical sympathetic ganglion of yearling calf. × 900.

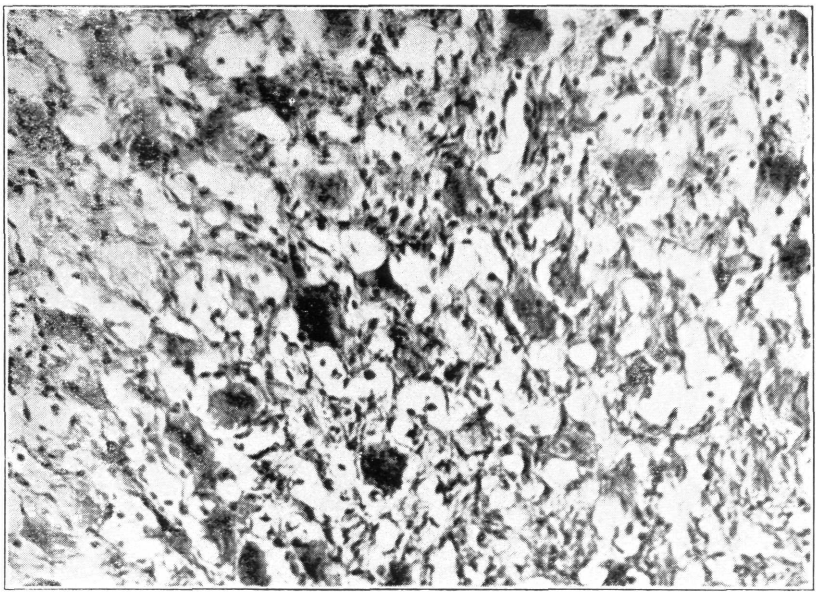

FIG. 4. Section of inferior cervical sympathetic ganglion of a one-year-old vitamin A deficient calf. × 900.

All of these changes occurred in the ganglia studied though not to the same degree in all the cells. Figure 1 illustrates the appearance of normal spinal ganglion cells in our series. Comparison with Figure 2 shows the effects of the deficiency. Most of the changes named above are to be seen in the cells of this section. The area photographed is quite typical of the ganglia of deficient animals. Most noticeable are the great shrinkage and the loss of distinctness of cell and nuclear outlines.

TABLE I

EFFECTS OF VITAMIN A DEFICIENCY ON THE SPINAL GANGLIA

NORMAL CONTROLS			VITAMIN A. DEFICIENT 55 TO 64 DAYS; SHOWED LOSS OF SENSIBILITY AND FLACCID PARALYSIS		
L-2	L-3	L-4	L-2	L-3	L-4
7918	6148	7453	7072	5743	6424
8085	9333	8354	6456	3862	6536
8737	9444	8403	6218	5840	4942
		8355	4934	6001	7782
		8457	5131	5385	6466
			6687	5858	6259
			5985	5905	6142
			3044	5763	6965
			6412	7899	7142
			6151	.....	7070
			6395	.....	7186
			.....	.....	7173
Average 8246	8308	8204	5862	5806	6674
Percentage of cells lost.....	.....	.....	29%	30.2%	18.7%

ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Sum of Squares	Variance	F Value
Vitamin Content.....	1	6,862,981	6,862,981	37.88**
Ganglia.....	2	195,848	97,924	.54
Interaction.....	2	281,583	140,791	.78
Error (within classes).....	37	.....	181,178	.....

The actual total numbers of cells in the normal and deficient ganglia are shown in Table I. The average of the different ganglia show the considerable lowering of the total number of cells present in the deficient cases. The percentages indicate a loss of from 18.7% to 30.2%. That these percentages are significant from the statistical standpoint is shown in the Analysis of Variance included in the table. The analysis proves that the factor responsible for the loss is the lack of vitamin A and that

the differences between normal and experimental animals are not due to errors in counting or chance variation among the ganglia used. As a matter of fact, recounts of many of the ganglia showed that the total is accurate to within 100 cells.

A short time before these cell counts were completed Mellanby ('38) reported that there was an overgrowth of new bone about the foramina of the skull and presumably those of the vertebral column. He suggested that the degeneration found in nervous tissues of vitamin A deficient animals might well be due to pressure of the bone growth on the nerve fibers as they pass through the foramina or on the ganglia themselves if surrounded by bone as in the spinal column.

This appeared to this writer to be a valid criticism of earlier reports. In an attempt to confirm or refute Mellanby's theory observations were made on the ganglia of the sympathetic chain of vitamin A deficient animals. In this location no pressure is possible on either cells or fibers. The material used consisted of ten ganglia of the sympathetic trunk from yearling calves reared on a diet completely deficient in vitamin A. At the end of a year the deficient animals (two) were emaciated, one was completely blind and the other nearly so, and the germinal epithelium of the testes was practically destroyed. New bone growth was found at the optic foramen, that of the blind animal being constricted to about two millimeters in diameter. This, of course, is in line with Mellanby's findings referred to above.

Sections of the inferior cervical sympathetic ganglia showed only moderate degenerative changes in one animal (partially blind) but in the blind calf the changes were so extreme that scarcely any cells were found which could be judged normal. Photographs are reproduced herewith to illustrate the normal and deficient ganglia. No attempt was made to make counts of the ganglion cells due to the lack of discreteness of the ganglia and the enormous number of cells present. In view of the structural changes evident in the photographs it hardly seems necessary.

CONCLUSIONS

The evidence submitted in this report shows that lack of vitamin A causes highly significant reduction in the total number of ganglion cells in the white rat.

The evidence further shows that there is extensive destruction of cells in sympathetic ganglia also in vitamin A deficient calves.

These observations account for the nerve fiber degeneration already reported since death of the cell bodies would inevitably result in degeneration of their processes.

Finally, while bone growth may be a factor in causing degeneration, Mellanby's theory of bone pressure as the principal cause of degeneration in nervous tissue is not acceptable since it occurs in ganglia where pressure is not a factor.

ACKNOWLEDGMENTS

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